

THE CREATION OF A STABLE pH GRADIENT IN A NONELECTROLYTE BUFFER SOLUTION MIXTURE. THE USE OF THIS SYSTEM FOR THE ISOELECTRIC FOCUSING OF ALBUMIN AND HEMOGLOBIN

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16. Abstract The article discusses the creation of a stable pH gradient in a nonelectrolyte buffer solution mixture, and the use of this system for isoelectric focusing of albumin and hemoglobin. Figures in the article depict the dependence of the pH of a buffer mix on the concentration of organic solvents, and the isoelectric spectra of proteins in rabbit hemoglobin and human serum albumin.					
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THE CREATION OF A STABLE pH GRADIENT IN A NONELECTROLYTE BUFFER SOLUTION MIXTURE. THE USE OF THIS SYSTEM FOR THE ISOELECTRIC FOCUSING OF ALBUMIN AND HEMOGLOBIN

(Submitted by Academician V. A. Engel'garb, 19 Aug. 1973)

The electrophoresis of proteins in a pH gradient has recently /955* been used with great success. This method is usually called "isoelectric focusing" and has been known for a long time [1,2]. The difficulties in creating a stable pH gradient have limited the use of this method to single examples. In recent years the buffer mix ampholin has been created [3], making it possible to maintain a pH gradient for an unlimited time; moreover, to create the pH gradient long-term electrophoresis of ampholins is necessary. Ampholins are a mixture of polyamino-polycarboxylic acids with a molecular weight from 300 to 1000, possessing the ability to distribute themselves on electrophoresis between the cathode and the anode so that an acidic region is formed around the anode, which is converted almost linearly to an alkaline region near the cathode.

We have primarily proposed another method for creating a stable pH gradient resulting from the changes in the dielectric properties of the medium in which the electrophoresis is conducted [4] of a solution of some organic solvent, for example a glycerin solution, is placed in the path of the buffer ion mix, then on entering this zone the dissociation constant of the buffer electro-

* Numbers in the margin indicate pagination in the foreign text.

lytes changes, and therefore the pH on exiting this zone will take on its original value. Glycerin is immobile in an electrostatic field, therefore the pattern of pH distribution will be determined by the method of distributing the organic solvent. We assumed [4] that in this system the variability in dielectric permeability (dp) is active, however on further use we became convinced that in certain cases the use of complex formation between nonelectrolytes and buffer ions is also promising for obtaining pH gradients.

The influence of dielectric permeability can be demonstrated by combining Henderson's buffer equation with the well-known relationship of dp influence on the dissociation constant [5,6]. Thus we obtain the following expression

$$\text{pH} = \text{pK}^0 + \frac{Z_i Z_j e^2}{2,303 D (r_i + r_j) RT} + \log \frac{a_{\text{a}}}{a_{\text{s}}}, \quad (1)$$

where D is the dielectric permeability, $Z_i Z_j$ is the valence of the ions, $r_i r_j$ is the radius of the ions, R the Boltzman constant, $a_{\text{a}} a_{\text{s}}$ - the activities of the acid and salt correspondingly, and e the charge on an electron.

In Figure 1a the solution of the given equation is presented for the cases of acetate buffer in water-glycerin, water-ethanol and water-dioxane solutions. The influence of the dp on the activity of the ions was not considered. A linear dependence of dp on the concentration of the nonaqueous component of the mixture was assumed. As is evident from the figure, there is a satisfactory correspondence between the theory (solid line) and the experimental results (points). It follows from eq. (1) that the value of the dependence of the buffer solution pH on the dielectric permeability is determined by the values of the radius and valence of the ions. For ions of large dimensions, for example proteins, the second term of eq. (1) is insignificantly small and may be neglected. Actually we have found that the isoelectric points of

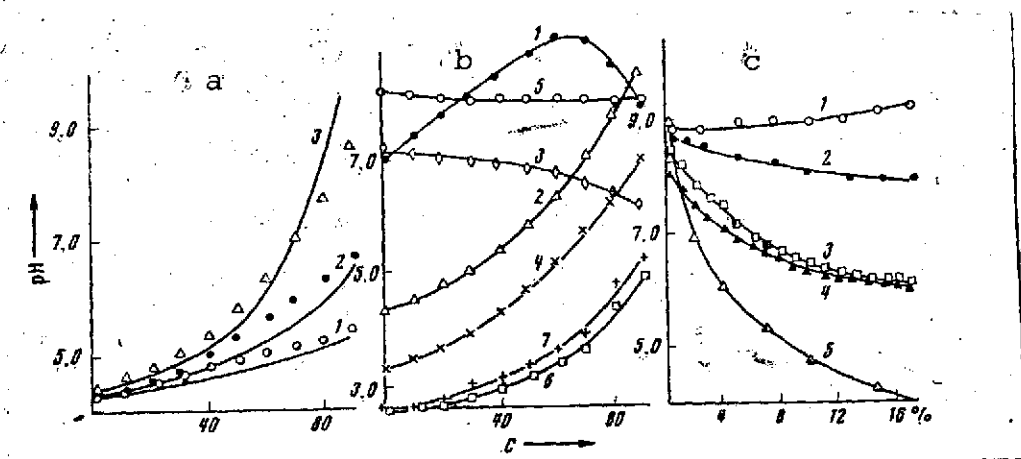
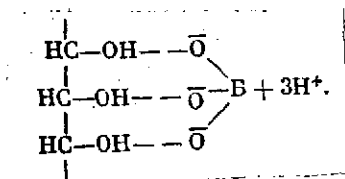


Fig. 1. The dependence of pH of a buffer mix on the concentration of organic solvents. a--0.00M acetate buffer on glycerin (1), ethanol (2), dioxane (3) (lines - theoretical calculations, points - experimental); b--different buffer mixes: 0.015M phosphate buffer (1), 0.002M citric acid and 0.0012M Na_2HPO_4 (2), 0.0072M triethanolamine and 0.0028M HCl (3), 0.007M formic and 0.002M NaOH (4), 0.01M tris-buffer (5), 0.01M formic acid (6), 0.01M acetic acid (7), on the concentration of dioxane; c-- 0.001M boro-borate buffer on the concentration of dioxane (1), sucrose (2), glycerin in 0.001M buffer (3), glycerin in 0.01M buffer (4), mannitol (5).

proteins in glycerin correspond to those in ampholins [4]. In Fig. 1b the results of a study of the dependence of the pH of different buffer mixtures and organic acids on the concentration of dioxane is demonstrated. As is evident from the figure, the character of the dependence is essentially determined by the nature of the buffer mix. The influence of complexation of buffer ions with substances added to change the dp_H is clearly evident from Fig. 1c. Here the experimentally obtained dependence does not correspond to the requirements of eq. (1). For dioxane

it is generally observed, while for sucrose, glycerin, and mannitol the relationship is inverted. Boric acid gives complexes with polyalcohols of the type



As a result of this it is strongly changed, as the action of the polyalcohol is determined by its conformation, and the complex is formed and destroyed with extreme rapidity [7], which was established using O_{18} . This data explains the large difference between the influence of sucrose on the one hand and glycerin mannitol on the other, obtained in our experiments (Fig. 1c). One may assume that the acidic cross-link in sucrose hinders its approach to boric acid, therefore its influence on the pH of boro-borate buffer is somewhat less than the influence of glycerin and mannitol. The practical knowledge of electrolyte-nonelectrolyte complex formation can be very significant for creation of pH gradients.

From the above it is evident that organic solvents and multi-atom alcohols can be used for creating pH concentration gradients, but it is necessary to consider that for different buffer mixes the mechanisms of formation of pH gradients may be different.

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According to our experiments, glycerin is the most expeditious for distribution and isoelectric focusing of proteins. On the one hand, this solvent does not practically influence protein conformation [8,9], while, on the other hand, the formation of glycerin concentration gradients leads to the development of a density gradient, which makes it possible to conduct isoelectric focusing in vertical columns. Aside from this, glycerine, which forms complexes with borate, makes it possible to create a pH range in boro-borate buffer up to 4.5 units wide.

—Such a gradient is stable for a long time (12

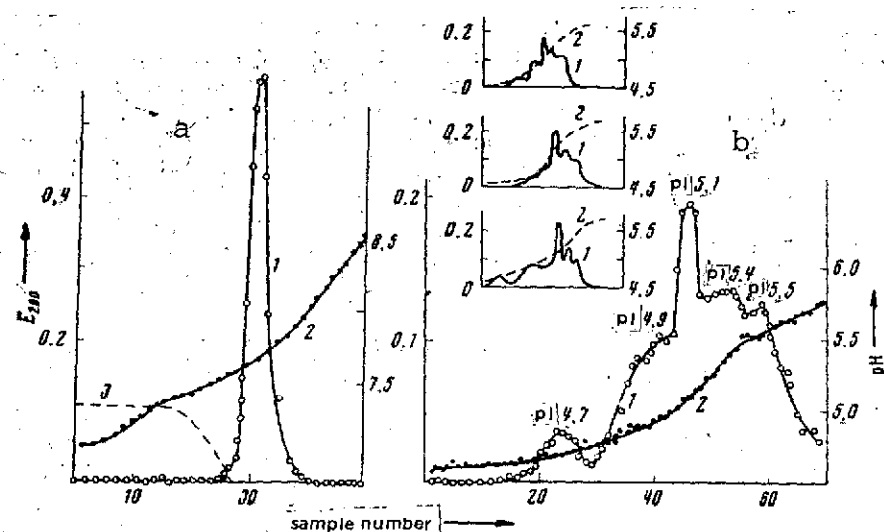


Fig. 2. The isoelectric spectra of proteins: a - rabbit hemoglobin, b - human serum albumin. In the inset - reproducibility in parallel experiments. 1 - absorption of protein fractions, 2 - pH gradient, 3 - distribution of the protein in the column before the experiment began.

day current passage). These wide gradients are not as practically important as the resolving power of the method is higher, if the zone of pH transition is stretched out on a large line. Isoelectric focusing was conducted in an apparatus which consisted of two vertically placed glass columns, the lower ends of which are united by a wide stopcock. The upper ends communicate with electrical receivers from a Tiselius electrophoretic apparatus by means of resin tubes. Let us consider the realization of the method using isoelectric focusing of human albumin as an example. As the starting buffer we took 0.001M acetate buffer pH 4.6. Using a gradient blender a glycerin concentration gradient from 0 to 90% was created in both columns. A pH gradient from 4.6 to 5.7 thus arose. The electrode vessels were filled with 0.1M

acetate buffer pH 4.6. The protein was layered on the density gradient in one of the columns. Isofocusing took three days at 4° and a voltage gradient of 10V/cm. At the end of the experiment the stopcock joining the columns was opened, and the solution was released in portions. The pH at absorption at 280mμ was determined in each sample and an isoelectric spectrum was constructed, which is presented in Fig. 2b. Here one may trace the experimental reproducibility with four parallel experiments as examples. Analogous data were obtained for ampholin [10]. In Fig. 2a the results of isofocusing of rabbit hemoglobin are presented. Forming a glycerin concentration gradient from 0 to 5% and simultaneously a sucrose concentration gradient from 0 to 30% (for obtaining stable gradient density) in 0.01M boro-borate buffer pH 8.6, a pH gradient from 8.6 to 7.0 was obtained. Here the results of a 12 day experiment are presented. Not considering the length of time of the experiment the pH gradient remains stable and smooth. The distribution of protein at the beginning of the experiment is shown by the dotted line. At the end of the experiment the protein concentration significantly increased. The concentration effect is determined by the value of the voltage gradient in the working part of the apparatus. /958

It is possible to indicate the following preferences for the method proposed by us as compared to ampholins. 1) The possibility of electrofocusing in a medium with a high ionic strength, which significantly extends the category of proteins and other ampholines which can be investigated by this method. 2) The possibility for electrofocusing low molecular weight substances of the peptide and amino acid types. The use of ampholins in this area is hindered due to the closeness of the molecular weight and structure of ampholins to amino acids and peptides. 3) The absence of need for preliminary electrophoresis to create

the pH gradient, which speeds up the work.

Standard equipment may be used to realize the proposed method. A pH gradient without ampholins may essentially be created in any columns from the firm "LKB" if glycerine or mannitol, rather than glucose, and the buffer system recommended by us, are used for creating the density gradient. It is possible to create a pH gradient in any given form by creating a given nonelectrolyte concentration gradient. In addition one may expect differences between the isoelectric focusing method in ampholins and in the buffer-nonelectrolyte system, due to complexation of protein with both ampholins and the nonelectrolyte substance.

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REFERENCES

1. G. V. Troitskiy, Elektroforez belkov (Protein Electrophoresis), Khar'kov, 1961.
2. M. Bier, Electrophoresis, N. Y., 1959.
3. H. Haglund, Isoelectric Focusing in pH Gradients, Stockholm, 1972.
4. G. V. Troitskiy, V. P. Zav'yalov, I. F. Kiryukhin, Bulletin of Experimental Biology and Medicine, Vol. 75, Issue 2, pg. 118, 1973.
5. E. A. Melvin-Kh'yuz, Fizicheskaya Khimiya (Physical Chemistry) 2, Foreign Literature Publishing House, 1962.
6. N. A. Izmaylov, Elektrokimiya Rastvorov (The Electrochemistry of Solutions), Khar'kov, 1959.
7. F. Kotton, G. Wilkinson, Sovremennaya Neorganicheskaya Khimiya, Ch. 2, M. (Contemporary Inorganic Chemistry, No. 2, Moscow), pg. 88, 1969.
8. I. F. Kiryukhin, G. V. Troitskiy, Ukr. biokhim. zhurn., Vol 39, No. 1, pg. 49, 1967.
9. I. F. Kiryukhin, Candidate's Dissertation, Sinferopol', 1965.
10. Nobuo Ui, Biochim. et biophys. acta, 229, 567, 1971.